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Athetis dissimilis (Lepidoptera: Noctuidae) is attracted to the sex pheromone of *Euzophera batangensis* (Lepidoptera: Pyralidae)

Junheon Kim [a], Bong-Kyu Byun [d], Hyun-Woo Oh [e], Sin Ae Jang [b], Chung Gyoo Park [a,b,c,*]

[a] Institute of Agriculture and Life Science, Gyeongsang National University, Jinju 52828, Republic of Korea
[b] Institute of Life Science, Gyeongsang National University, Jinju 52828, Republic of Korea
[c] Division of Applied Life Science (BK21+ Program), Gyeongsang National University, Jinju 52828, Republic of Korea
[d] Department of Biological Sciences and Biotechnology, Hannam University, Daejeon 34430, Republic of Korea
[e] Korea Research Institute of Bioscience and Biotechnology, Daejeon 34141, Republic of Korea

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ABSTRACT

Euzophera batangensis (Lepidoptera: Pyralidae) is one of the troublesome pests in sweet persimmon trees due to the larvae which are feeding under the bark. *Athetis dissimilis* (Lepidoptera: Noctuidae) was first reported as a pest of summer maize seedlings in Shandong province in 2014. Afterwards, it is rapidly spreading its distribution to several areas in China. While conducting some field experiments using the sex pheromone of *E. batangensis* in sweet persimmon orchards, a number of *A. dissimilis* was caught unexpectedly to the traps baited with the sex pheromone of *E. batangensis*, (Z)-9-tetradecan-1-ol (Z9-14OH) and (Z9,E12)-tetradeca-9,12-dien-1-ol (Z9,E12-14OH). Sequence BLAST search in the BOLD database diagnosed the moths as *A. lepigone*. However, morphological analysis definitely identified the moths as *A. dissimilis* by difference in male genitalia from that of *A. lepigone*. Catches of *A. dissimilis* to the single component of Z9-14OH or Z9,E12-14OH, and their 1:9 binary blend showed that the moth occurred twice a year, mainly from May 28 to June 25 and from August 13 to September 3. More number of *A. dissimilis* was attracted to 1:9 binary blend than each single component, with no significant difference among the pheromonal attractants. Thus, the 1:9 binary blend of Z9-14OH and Z9,E12-14OH can be used as a co-attractant for *E. batangensis* and *A. dissimilis*.

Introduction

Persimmon (*Diospyros kaki* Thumb.) is one of the economically important tree fruits in Korea, China, and Japan. Among the pests damaging persimmon trees and fruits, bark-feeding moths such as *Synanthedon bicingulata* (Staudinger) (Lepidoptera: Sessidae) and *Euzophera batangensis* Caradja (Lepidoptera: Pyralidae) have been recently arisen as one of troublesome insect pests in Korea (Park et al., 2015).

(Z9,E12)-Tetradeca-9,12-dien-1-ol (Z9,E12-14OH) and (Z)-9-tetradecen-1-ol (Z9-14OH) were identified from the sex pheromone gland extract of *E. batangensis* and they evoked electro-antennal activities suggesting possibility of the components as sex pheromone of *E. batangensis* (Kalinová et al., 2006). In fact, the Z9,E12-14OH and Z9-14OH showed attractiveness toward *E. batangensis* singly and in binary blend in the field in China (Wen et al., 2009). In China, the occurrence pattern and integrated control technology were studied for *E. batangensis* (Huang, 1995). However, no other information is available in Korea and China.

We performed some experiments on field trapping of *E. batangensis* and on efficacy of the sex pheromone components and trap types. During these experiments, a lot of non-target species was attracted to the sex pheromone traps of *E. batangensis*. This unexpected insect species were identified by morphological and DNA barcode investigations as *Athetis dissimilis* (Hampson) (Lepidoptera: Noctuidae). *A. dissimilis* was recently reported as a pest attacking summer maize seeding in China (Li et al., 2014).

We report herein the morphological characteristics, DNA sequencing results, and annual occurrence pattern of *A. dissimilis*, and the attractiveness of sex pheromone of *E. batangensis* to *A. dissimilis*.

Materials and methods

Taxon sampling

Materials examined in this study were a part of the non-targeted catches by the sex pheromone traps of *E. batangensis* in sweet persimmon orchards in Jinju, Sancheong, and Sacheon, Republic of Korea, 2015.

* Corresponding author at: Institute of Life Science, Gyeongsang National University, Jinju 52828, Republic of Korea.
E-mail address: parkcg@gnu.ac.kr (C.G. Park).

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Morphological identification

Materials examined herein were preserved in the Entomological Collection of Department of Biological Science & Biotechnology, Hannam University (EHNU; Dajeon, Republic of Korea) and Gyeongsang National University (Jinju, Republic of Korea). For the morphological examination, external characters including wing pattern and male genitalia were examined with stereo-microscope (ZEISS 2000-C, Carl Zeiss AG, Göttingen, Germany).

DNA extraction, amplification and sequencing

DNA was extracted from a leg of dried specimens using a DNeasy kit (Qiagen, Hilden, Germany) following the manufacturer's protocol, except that the final elution step was performed with 60 μL of Milli-Q water instead of 200 μL buffer. All of the vouchers from this study are deposited in Hannam University and Gyeongsang National University. A 658-bp segment of the barcode region was amplified from specimens using the primer listed in Table 1 (Hajibabaei et al., 2006). PCRs were performed using a Maxime® PCR PreMix (iNtRON Biotechnology, Seongnam, Republic of Korea) with 2.0 pmol of each primer and 2–50 ng of template DNA in a 20 μL reaction. PCR thermocycling was done under the following conditions: at 95 °C for 2 min; 5 cycles at 94 °C for 40 s, at 45 °C for 40 s, at 72 °C for 60 s; 40 cycles at 94 °C for 40 s, at 51 °C for 40 s, at 72 °C for 60 s; and final extension at 72 °C for 5 min. PCR products were visualized in a 2% agarose gel stained with ethidium bromide and bidirectionally sequenced using a BigDye Terminator ver. 3.1 Cycle Sequencing Kit (Applied Biosystems Inc., Foster, CA) on an ABI 3730XL capillary sequencer. Contigs were assembled using CodonCode Aligner ver 3.5.6 (CodonCode Co., Dedham, MA) and were subsequently aligned using the same software or MEGA (Tamura et al., 2013). Sequence divergences were calculated using the Kimura 2-parameter (K2P) model (Kimura, 1980).

Chemicals

Pheromone components of *B. batangensis*, (Z)-9-tetradecen-1-ol (Z9-14OH; 96% pure) and (Z9,E12)-tetradeca-9,12-dien-1-ol (Z9,E12-14OH: 96% pure), were purchased from Bedoukian Inc. (Danbury, CT, USA).

Pheromone lures

Pheromone lures were prepared by loading the pheromone components (0.5 mg) dissolved in hexane to an 11-mm red rubber septum (Wheaton Scientific, Millville, NJ, USA). Pheromone component was Z9-14OH, Z9,E12-14OH and with their 1:9 mixture.

Field bioassay

Two field trapping experiments were conducted from April 30 to October 29, 2015. For each field trial, traps were set in randomized complete block design with 3 replications. The distance between the traps within a replicate was at least 10 m. Each block was apart from at least 20 m. Numbers of moth catches were checked, sticky plates were changed newly, and the uni-traps were cleaned every week. The pheromone lures were renewed every 4 weeks.

Table 1
PCR primers used in this study.

Primer name	Primer sequence (5′–3′)
LepF1	ATTCAACCAATCATAAAGATATTGG
LepR1	TAAACTTCTGGATGTCCAAAAAATCA
MLepF1	GCTTTCCCACGAATAAATAATA
MLepR1	CCTGTTCCAGCTCCATTTTC

Primer source: Hajibabaei et al. (2006).

Attractiveness of Z9-14OH, Z9,E12-14OH, and their 1:9 mixture

The uni-traps (Green Agro Tech; Gyeongsan, Republic of Korea) containing the pheromone components of *B. batangensis* (Z9-14OH and Z9,12E-14OH, singly or 1:9 mixture) were installed at two sweet persimmon orchards in Jinju (Jinju A; 35°08′29.92″N, 128°08′13.04″, Jinju B; 35°09′32.15″N, 128°10′02.57″E). As a control, a red rubber septum which only hexane was applied was used.

Efficacy of trap types

Efficacy of 3 different trap types (delta traps with white or red color, and uni-trap; Green Agro Tech) was investigated at sweet persimmon orchard in Sancheong (35°18′31.59″N, 127°58′54.43″E) and Sacheon (35°03′25.78″N, 128°05′33.20″E). As a lure, Z9,E12-14OH (0.5 mg) was used.

Statistics

The number of males caught (X) was transformed to log ($X + 1$) and analyzed by analysis of variance (ANOVA). Before the statistical analysis, the Shapiro–Wilk test was used to check for normality distributions. Means were compared using the Tukey–Kramer HSD test (JMP ver. 9.01; SAS Institute, Cary, NC). The numbers of *A. dissimilis* captured are shown in the Results section as the mean ± standard error of mean (SEM). For analysis of annual occurrence pattern, the numbers of *A. dissimilis* captured in the same site was pooled.

Results

Taxonomic accounts
Order Lepidoptera
Family Noctuidae
Athetis dissimilis (Hampson, 1909)
Proxenus dissimilis Hampson, 1909, Cat. Lepid. Phalaenae Br. Mus. 8: 431, pl. 133: 15 (TL: Japan (BMNH, London))

Diagnosis. This species is very similar with *A. lepigone* (Möschler, 1860). But it can be distinguished by the male genitalia with rather broad and rectangular valve, slightly emarginated outwards laterally (Fig. 1) (Kononenko & Han, 2007).
Adult. Wingspan 24–29 mm in male. Ground color of forewing blackish brown with no pattern. In hindwing, grayish white, apex rounded, rather darkened along costa and termen.
Male genitalia. Tegumen rounded. Valva expanded with rather broad and rectangular beyond middle, numerous hairs along the apex of valve, slightly emarginated medio-laterally, strongly sclerotized from base to middle with a spine at its end. Aedeagus long enough 2/3 of valve, with two cornuti in vesica.
Material examined. 22♂, Jinju, GN (pheromone trap), 3. VI. 2015 (leg. JG Park)-gen. slide. No. 4063, 5191–5194, 5208–5210-coll. EHNU.
Distribution. Korea (Central, South, Jeju), Japan, China, Taiwan, the Philippines, Indonesia.

DNA extraction, amplification and sequencing

Four 658 bp sequences and fifteen 407 bp sequences in total from the 19 COI barcodes were obtained from 19 dried moth specimens. Molecular identification result on the BOLD system (http://www.boldsystems.org/) showed 18 sequences were matched to *A. lepigone* with a sequence similarity of 99.25–99.5% (17 samples) and 100% (one sample). The rest one was identified as *A. putris* (Lepidoptera: Noctuidae) with a sequence similarity 100%. The K2P distance of the two species *A. dissimilis* and *A. lepigone* was about 12%.